

Amendments to the Specification

Please amend the paragraph beginning at page 37, line 10, as follows:

The DNA sequence encoding human amine receptor, ATCC #97181, is initially amplified using PCR oligonucleotide primers corresponding to the 5' and 3' end sequences of the processed amine receptor nucleic acid sequence (minus the signal peptide sequence). Additional nucleotides corresponding to amine receptor gene are added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence 5' CGGAATTCCTUATGAGAGCTGTCTTCATC 3' (SEQ ID No. 3) contains an EcoRI restriction enzyme site followed by 18 nucleotides of human amine receptor coding sequence starting from the presumed terminal amino acid of the processed protein. The 3' sequence 5' CGGAAGCTTCGTCATTCTTGGTACAAATCAAC 3' (SEQ ID No. 4) contains complementary sequences to an HindIII site and is followed by 18 nucleotides of the human amine receptor gene. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc. Chatsworth, Calif.). pQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. pQE-9 is then digested with HindIII and EcoRI. The amplified sequences are ligated into pQE-9 and are inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture is then used to transform *E. coli* strain M15/rep 4 (Qiagen, Inc.) by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis. Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 µg/ml) and Kan (25 µg/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.⁶⁰⁰) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl.

After clarification, solubilized human amine receptor is purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). Human amine receptor protein is eluted from the column in 6 molar guanidine HCl pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100 mM sodium phosphate, 10 mmolar glutathione (reduced) and 2 mmolar glutathione (oxidized). After incubation in this solution for 12 hours the protein is dialyzed to 10 mmolar sodium phosphate.

Please amend the paragraph beginning at page 42, line 20, as follows:

The DNA sequence encoding Human amine receptor, ATCC #97181, is constructed by PCR using two primers: the 5' primer 5' GTCCAAGCTTGCCACCATGAGAGCTGTCTTCATC 3' (SEQ ID No. 7) contains a HindIII site followed by 18 nucleotides of Human amine receptor coding sequence starting from the initiation codon; the 3' sequence 5' CTAGCTCGAGTCAAGCGTA GTCTGGGACGTCGTATGGGTAGCATTCTTGGTACAAATCAAC 3' (SEQ ID No. 8) contains complementary sequences to an XhoI site, translation stop codon, HA tag and the last 18 nucleotides of the Human amine receptor coding sequence (not including the stop codon). Therefore, the PCR product contains a HindIII site, human amine receptor coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag, and an HindIII site. The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with HindIII and XhoI restriction enzymes and ligated. The ligation mixture is transformed into *E. coli* strain SURE (Stratagene Cloning Systems, La Jolla, Calif.) the transformed culture is plated on ampicillin media plates and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant amine receptor, COS cells are transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the Human amine receptor HA protein is detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells are labelled for 8 hours with ³⁵S-cysteine two days post transfection. Culture media is then collected and cells are lysed with detergent (RIPA buffer (150 mM

NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50 mM Tris, pH 7.5) (Wilson, I. et al.,
Id. 37:767 (1984)). Both cell lysate and culture media are precipitated with a HA specific
monoclonal antibody. Proteins precipitated are analyzed on 15% SDS-PAGE gels.
